T- and B-Cell Clonality Evaluation in Primary Cutaneous Lymphomas Using Standardized BIOMED-2 PCR Protocols

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INTRODUCTION

Polymerase chain reaction (PCR)-based methods in the detection of T-or B-cell receptor gene rearrangements have become routine and reliable techniques in the assessment of lymphoid clonality in clinical samples. Recently, an international consensus primers for both T-cell receptor (TCR) and immunoglobulin heavy chain (IgH) gene rearrangements (BIOMED-2 Concerted Action BMH4-CT98-3936 project) have been proposed and new techniques using high-resolution automated fluorescent scanning systems for analyzing PCR fragments have been introduced.

The aim of this study was to determine the usefulness in clonality detection of the BIOMED-2 Concerted Action BMH4-CT98-3936 project standardized TCR/IgH PCR primers and protocols in paraffin-embedded samples in a series of primary cutaneous T- and B-cell lymphomas (CTCL/CBCL). The PCR products were run on a capillary electrophoresis system and analyzed using GeneScan software (GS).

METHODS

One hundred and seventy one paraffin-embedded samples obtained from patients diagnosed between 2001 to 2005 in different hospitals from the Catalonian Cutaneous Lymphoma Network (Barcelona, Spain) were evaluated. All cases fulfilled the inclusion standardized WHO-EORTC classification criteria for the diagnosis of T- and B-cell primary cutaneous lymphomas: 105 biopsies from patients with CTCL were included in the study, corresponding to 51 patch/plaque stage mycosis fungoides (MF), 3 tumor stage MF, 3 Sézary syndrome (SS), 29 patients presenting lymphomatoid papulosis (LyP), 6 primary cutaneous large cell CD30+ lymphoma and 13 non-MF CTCL cases. In addition, 29 cases, clinically consistent with early patch stage MF but without evidence of histopathologically diagnostic features of MF, were diagnosed as "large-plaque parapsoriasis" (PP) and included in the study. Thirty-seven unequivocal cases of primary CBCL: 27 marginal zone (MZL) showing monotypic kappa or lambda light chain restriction and 10 cutaneous large B-cell lymphomas (LBCL) were evaluated. Thirty samples corresponding to inflammatory disorders (psoriasis/eccema) and 18 samples diagnosed of lymphoid reactive hyperplasia (LRH) were also analyzed.

Clonality studies

DNA was obtained from two 15 micrometer sections of paraffin-embedded biopsies using the QIAamp Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer instructions. T-cell clonality was evaluated by PCR amplification with specific oligonucleotides for the TCRγ as previously described. TCRβ gene rearrangements were studied in cases showing no clonal rearrangement for the TCRγ (Figure 1). IgH gene rearrangement was amplified by PCR with consensus specific primers for the complete rearrangements VDJ analyzing framework region (FR)1, FR2 or FR3 (Figure 2) and for incomplete DJ rearrangements of the IgH gene with the standardized BIOMED-2 PCR protocol. Ig kappa light chain (IgK) and rearrangements involving the kappa deleting element (KdE) were performed in cases non-conclusive for the IgH rearrangements (Figure 3). All PCR amplifications were performed in duplicate for each skin biopsy to avoid the false-positive interpretation of monoclonality (pseudomonoclonality).

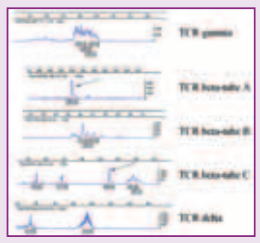

Figure 1

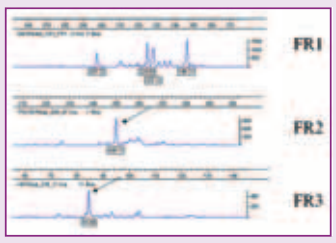

Figure 2

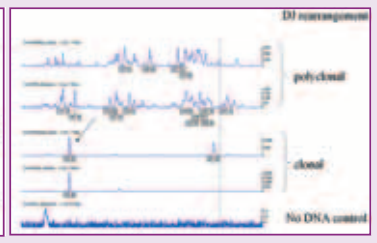

Figure 3

RESULTS

A dominant T-cell clone was demonstrated in 63% of CTCL cases by means of TCRgamma analysis: 34/51 (66%) patch/plaque stage MF, 2/3 (66%) tumor MF, 2/3 (66%) SS, 15 out of 29 (52%) skin biopsies of LyP lesions, in 4/6 (66%) of CD30+ lymphoma cases and 9/13 (69%) of non-MF CTCL. T-cell clonality was identified in only 1/29 (3%) of PP and none of the benign chronic inflammatory samples revealed a dominant clone. TCRβ analysis detected additional monoclonal results in 3 MF, 1 panniculitis-like TCL and 1 SS samples that showed no informative results after TCRγ amplification. Using this combined approach overall T-cell clonality detection rate in paraffinized MF patch/plaque samples was 71%.

B-cell monoclonality detection was demonstrated in 73% of CBCL cases (18/27 (67%) MZL and 9/10 (90%) of LBCL) using a combined approach of FR1, FR2 and FR3 primers of the IgH gene. When analyzing exclusively FR3 region, only in 43 % of MZL cases a B-cell monoclonal population was identified. The addition of primers directed to incomplete DJ rearrangements and Ig kappa rearrangement, identified 6 additional clonal cases by PCR analysis in the MZL group (overall B-cell monoclonality rate of 89% in MZL). None of the LRH samples disclosed a dominant clone. Results are summarized in Tables 1 and 2.

Table 1

CTCL type	n	TCR gamma	TCR beta	Frozen sample
MF patch/plaque	51	34 (66%)	2/17	4/6 (66%)
MF tumor	3	2 (66%)	1/1	6/10 (60%)
PP	29	1 (3%)		
SS	3	2 (66%)	1/1	
CD30 + CTCL	6	4 (66%)		
LyP	29	15 (52%)		
CTCL other	13	9 (70%)	1/4	
Psoriasis/der.	30	1 (3%)		

Table 2

CBCL type	n	IgH (%)	FR3	FR2	FR1	DJ	IgK	KdE
Marginal zone	27	22 (81%)	8/19 (43%)	8/15 (53%)	8/11 (73%)	4/9 (44%)	1/5 (20%)	2/9 (22%)
Follicle centre CBCL	3	3 (100%)	2/3	3/3	1/3	-		
CBCL leg type	7	6 (86%)	5/7	3/5	4/5	0/1		
Secondary CBCL	4	4 (100%)	3/4	3/$	2/2			
Pseudolymphoma	18	0 (0%)	0/18	0/13	0/13			
Inflammatory der.	9	0 (0%)	0/9	0/3	0/3			

COMMENT

The demonstration of a monoclonal cell proliferation in T- or B-cell atypical cutaneous lymphoid infiltrates has become an important criterion for the support of a diagnosis of cutaneous lymphoma, especially when morphological and immunophenotypical features are non-conclusive.

Primers directed against FR3 region of the various VH genes for the PCR analysis of the IgH chain are the most widely applied to B-cell clonality studies in pathology laboratories. According to the PCR strategy employed in our series, the false negative rate for B-cell clonality testing by PCR of the FR3 region can be as high as 50% when paraffin-embedded tissue samples are studied. Nevertheless, the addition of other FR region primers will increase the detection rate of this test. False negative detection rate of B-cell clonality seems to be significantly reduced using additional primers directed to incomplete DJ and Ig kappa rearrangements.

Our study shows that BIOMED-2 PCR approach and GS analysis of TCR/IgH rearrangements appeared to be a highly informative strategy in the diagnostic process of patients with a suspicion for primary CTCL/CBCL. Our results demonstrate that this method is reliable and useful, detecting a high percentage of clonality, when analyzing paraffinized material. It seems to be particularly informative and specific for the distinction of early MF and benign inflammatory chronic dermatosis.

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